Impact of Varied Lead Concentrations on Lead Accumulation in the Body, Heart and Gill Damage of Milkfish (*Chanos chanos*)

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Abstract

Milkfish (Chanos chanos) is valued in aquaculture for its affordability and nutritional richness. However, conventional farming techniques frequently result in suboptimal yields due to lead (Pb) contamination. Traditional milkfish ponds are typically situated near coastal areas, river waters, estuaries, ports, and industrial zones, rendering them susceptible to contamination, particularly from heavy metals, like Pb. This study aimed to analyze Pb accumulation in the body of milkfish juvenile, identify liver and gills damage, and examine the growth rate and survival of milkfish exposed to Pb at various concentrations. Juvenile milkfish were exposed to various concentrations of PbNO3, namely 0 mL.L^{-1}, 0.08 mL.L^{-1}, 0.8 mL.L^{-1}, and 8 mL.L^{-1}, including the non-exposed control group. The metal content of the fish bodies was measured using an AA spectrophotometer. Histological analysis of the liver and gills of the fish was performed to evaluate organ damage. The results showed significant Pb accumulation has been observed in milkfish exposed to high Pb concentrations. The control treatments showed a decrease in metal content, whereas treatments with high concentrations showed a significant increase. Exposure to Pb within 30 d can cause organ damage, such as inflammatory cells, secondary lamella fusion, and necrosis that occurs in the gills. In the liver, there is damage such as the accumulation of inflammatory cells, necrosis, and hydrofic degeneration. Pb exposure rapidly damages and disrupts milkfish's biological functions, influencing survival and growth. Pb exposure with doses 8 mL.L^{-1} significantly affected juvenile milkfish within approximately 60 min.

Keywords: Gill damage, Lead, Liver damage, Metal accumulation, Milkfish

Introduction

Milkfish (*Chanos chanos*) is a superior aquaculture commodity that is popular in the community owing to its affordable price and high nutritional value. Unfortunately, milkfish farming often uses traditional farming systems, resulting in low productivity. Traditional milkfish ponds are usually located around coasts, river waters, estuaries, ports, and industrial areas, making them vulnerable to heavy metal pollution, especially Lead (Pb) (Awaluddin *et al.*, 2020). Lead (Pb) is a dangerous heavy metal that can pollute water and negatively impact aquatic organisms. These metals can enter the body of fish through food, respiration, and the digestive tract, causing oxidative stress, reproductive disorders, and biochemical changes (Zulfahmi *et al.*, 2021). Pb pollutants originate from small boats, fishing boats, fossil fuels, pesticides, and diesel-fueled tractors (Widowati *et al.*, 2022). Water quality standards stipulate that the lead content in water and fish should be low (0.08 mg.L^{-1} and 0.3 mg.kg^{-1}, respectively).

High Pb concentrations can accumulate in fish and shellfish, potentially killing these organisms (Jais *et al.*, 2020; Afiyatillah *et al.*, 2022; Apresia *et al.*, 2023). Heavy metals, such as lead, are non-degradable; therefore, their accumulation in organisms can negatively impact them in the long run. Algae that grow in sediments can also take lead, which becomes food for milkfish (Khairuddin *et al.*, 2021). Long-term exposure to Pb can cause organ and tissue damage, including mutagenesis, carcinogenesis, teratogenesis, and liver damage (Adegbola *et al.*, 2021). The liver is an essential organ for the detoxification of chemicals, and damage can occur if exposure continues. Gills are the primary sites for oxygen uptake and are sensitive to changes in water quality, which can lead to severe stress (Sathick *et al.*, 2019). Histological studies have evaluated its toxic effects on fish organs including the

liver and gills. Analysis of heavy metals in fish tissues is critical because they can cause significant health risks if they accumulate for too long.

This study aimed to analyze Pb accumulation in the juvenile body of milkfish, identify organ damage (in the liver and gills), and examine the growth rate and survival of milkfish exposed to various Pb concentrations.

Materials and Methods

This study was conducted by exposing $PbNO_3$ to water, a fish-growth medium. Water was exposed to PbNO3 at different concentrations (0, 0.08 mL.L^{-1}, 0.8 mL.L^{-1}, and 8 mL.L^{-1}, and the experiment was repeated thrice. The acclimatization stage of the test fish lasted for six days in a pond measuring 100x60x40 cm, with 25 ppt salinity seawater equipped with an aeration system. During the acclimatization period, the test fish were fed pellets twice daily.

Stocking of test animals

The test animals were weighed before being put into the aquarium to determine their initial weight using a Vernier type VEB 2000 C digital scale with an accuracy of 0.01 gr. The test animals were placed in an aquarium containing lead-exposed water. The aquarium measured $40 \times 36 \times 32$ cm^3 and was filled with 30 L of seawater with a salinity of 25 ppt. Thirty test fishes were placed in each aquarium.

Maintenance and observation

The exposure period was 30 d. During the maintenance period, juveniles were fed as much as 10% biomass per day, with a feeding frequency of three times daily *i.e.* at 07.00, 12.00 and 17.00. Water was changed by siphoning three times a day, namely before feeding, and once every ten days, the water was changed by 80%. The water used for water replacement is water that has been added with $PbNO_3$ according to the concentration of each treatment. Fish are sampled every ten days to track the progression of their weight during maintenance. Additionally, daily assessments are conducted on water quality, focusing on parameters such as temperature, dissolved oxygen (DO), salinity, and pH.

Metal content in the body of milkfish

The metal content in wet milkfish samples was analyzed by first placing the samples in a glass beaker. Subsequently, 10 ml of nitric acid was added to the beaker. The beaker was then positioned in a water bath and heated until the milkfish sample was thoroughly dissolved and clarified. Following this, the solution was filtered using filter paper. To establish a calibration curve linking measured light intensity to metal concentration, a standard solution with a known metal concentration was prepared. The milkfish sample was then introduced into the compartment of a spectrophotometer, and the absorbance of light passing through the sample was recorded. Analysis of the metal content in the milkfish body was conducted using an AA Spectrophotometer.

Histology

The organs or samples were fixed in 10% neutral-buffered formalin before further processing and embedding in paraffin wax. Sections 7 mm thick were cut using a rotator microtome and stained with hematoxylin and eosin to study histology and cellularity. Gradual dehydration was performed using a series of alcohol solutions of gradual concentration, followed by immersion in xylol. The deparaffinization stage involves a series of reverse processes from xylol to alcohols of different concentrations, namely 70%, 80%, 90%, and 95%, and finally to water. Then, Hematoxylin-Eosin (HE) staining was performed to visualize cell and tissue structures. This staining provides a good contrast between the cell nucleus (blue) and the cytoplasm and other cell structures (red or pink), facilitating the identification and evaluation of cell and tissue morphology. After HE staining for 10 min and eosin staining for 20 min, the samples were clamped with a coverslip using Entellan. The final image is observed under a microscope and photographed for histological analysis.

To measure growth, the test animals were every ten days for 30 d during maintenance. We observed the animals by sampling and selecting ten individuals per aquarium. Growth was calculated using the Effendi formula (1997). Survival observations were performed daily to determine the survival of test animals

Data analysis

To determine the effect of Pb on metal accumulation in the body, milkfish's growth and survival were analyzed using analysis of variance (ANOVA). If there is a significant effect, we proceed with the W-Tukey test. The SPSS program package was used to carry out statistical tests. Organ damage (gills and liver) was analyzed descriptively.

Result and Discussion

The content of metals in the body

The results of the variety analysis showed that the concentration of Pb in water during the maintenance of juvenile milkfish larvae had a

significant influence on Pb content in the milkfish body. Furthermore, the results of the W-Tukey test showed that the Pb metal content in the milkfish body in treatment A (0 mL.L^{-1}) was not significantly different from treatment B (0.08 mL.L^{-1}) ($P>$ 0.05) but very different from treatment C (0.8 mL.L^{-1}) and D (8 mL.L^{-1}).

Based on the picture above, the results of measuring the Pb metal content of treatment A (0 mL.L^{-1}) from the beginning of observation to the end decreased drastically from 0.492 to 0.003 mg.g^{-1}. In treatment B (0.08 mL.L^{-1}), the Pb metal content in the body of milkfish juveniles also tended to decrease, at the beginning of the maintenance of milkfish juveniles containing as much as 0.492 mg.g^{-1} Pb. On day 15, maintenance decreased to 0.413 mg.g^{-1}; at the end of the observation period, it also decreased to 0.073 mg.g^{-1}. In treatment C (0.8 mL.L^{-1}), the Pb metal content in the juvenile body of milkfish increased from the beginning of maintenance, from 0.492 mg.g^{-1} to 3.077 mg.g^{-1} on day 15, and also increased at the end of observation, which was 3.207 mg.g^{-1}. In treatment D (8 mL.L^{-1}), the metal content in the juvenile milkfish body increased drastically from 0.492 mg.g^{-1} to 6.813 mg.g^{-1}.

The results of measuring Pb metal content from the first day to the thirtieth day in treatment A (0 mL.L^{-1}) found that the metal content decreased by 0.489 mg.g^{-1}. In treatment B (0.08 mL.L^{-1}) the metal content in the juvenile body of milkfish decreased by 0.419 mg.g^{-1}, whereas in treatment C (0.8 mL.L^{-1}) the Pb metal content in the juvenile body of milkfish increased by 2.715 mg.g^{-1}, and treatment D also increased Pb by 6,321 mg.g^{-1}, suggesting that the concentration of Pb in water is related to the metal content in the juvenile body of milkfish. Fluctuations in the metal content in the bodies of milkfish were directly proportional to the concentration of Pb in the maintenance container. In treatments A (0 mL.L^{-1}) and B (0.08 mL.L^{-1}), the metal content was significantly decreased. In contrast, in treatments C (0.8 mL.L^{-1}) and D (8 mL.L^{-1}), there was a significant increase in metal content, which was influenced by the Pb concentration in the maintenance container. The metal content in the juvenile body of milkfish and the Pb concentration in the water were positively correlated. If the concentration of Pb is high, the Pb content in the body of the milkfish is also high, and vice versa. Fish can absorb metals directly from the surrounding water or indirectly from other organisms such as small fish, invertebrates, and aquatic vegetation (Kumar and Nandan, 2020).

Exposure to Pb metal at different concentrations showed a noticeable influence on Pb concentration in the body of milkfish juveniles ($P<0.01$). Based on the above graph, in treatment A (0 mL.L-1), the Pb metal content decreased from the maintenance beginning and obtained the lowest metal content, maybe because treatment A (0 mL.L^{-1}) did not contain Pb. In contrast to the treatments with C (0.8 mL.L-1) and D (8 mL.L-1), whose body metal content increased dramatically from the beginning of exposure to Pb, milkfish juveniles could absorb the metal around them.

Pb concentration in the fish body decreased at concentrations of 0 and 0.08 mL.L^{-1}. This is probably because the concentration of Pb exposed is very low. This decrease is believed to be attributable to the low concentration of exposed Pb. Metabolism or biological transformation of heavy metals can effectively mitigate heavy metal toxicity. When heavy metals enter the plant body, they undergo binding and a subsequent reduction in toxicity, as they are transformed into simpler forms of compounds. This process is facilitated by enzyme activity, which regulates and accelerates the transformation. Through this mechanism, the concentration of heavy metal toxins in marine waters can be diminished and neutralized (Setiawan, 2014).

Metal accumulation in the tissues of organisms living in aquatic ecosystems is generally influenced by several factors such as the duration of exposure to metals, type of metal, metabolic activity in tissues, and physical and chemical properties of water (such as pH, temperature, salinity, and hardness). Similarly, metal accumulation in fish tissues depends on metal absorption, distribution, deposition, and excretion (Al-Kshab and Yehya, 2021).

Table 1. The average Pb metal content in the juvenile body of milkfish exposed to Pb with various concentrations

Pb concentration (mL.L^{-1})	Average changes in metal content in the body ± SD
A (0)	0.489±0.006[a]
B (0.08)	0.419±0.015 [a]
C (0.8)	2.715±0.617 [b]
D (8)	5.710±2.625[c]

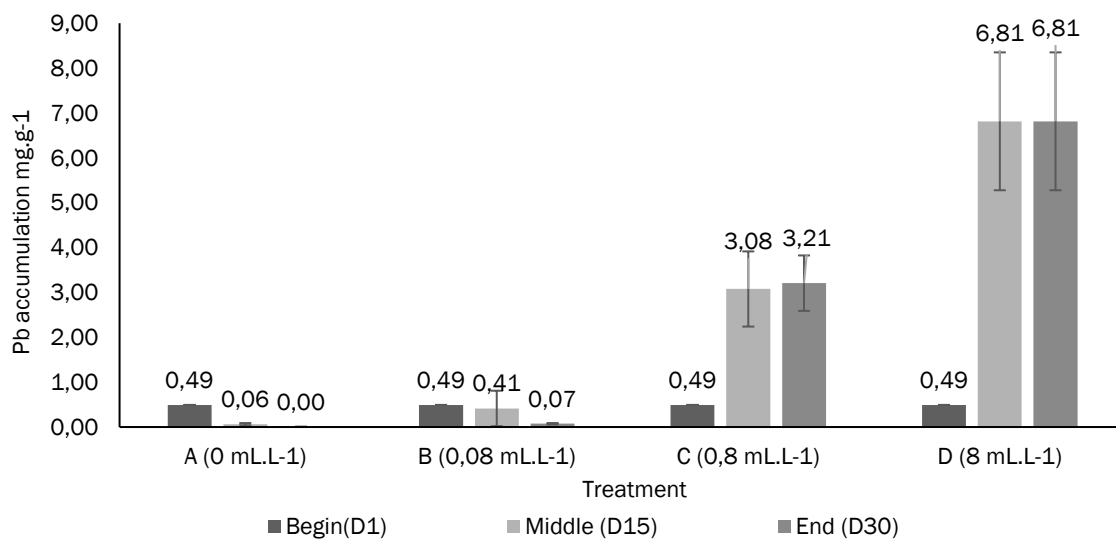Note: Different letters in the same column indicate significant effects ($P<$ 0.05).

Figure 1. Pb metal content in juvenile milkfish bodies

Heavy metals enter fish bodies in three potential ways: by gills, by the body surface, and by the digestive tract. Pb enters the body of fish in several ways, either directly through the digestive tract by consuming contaminated water and food or indirectly through the gills or skin. Some literature also suggests that heavy metals such as Pb can enter the fish body through various pathways, including the gills, body surface, and gastrointestinal tract (Sonone *et al*., 2020). Metal uptake from water is directly carried out by the gills, which is consistent with research findings showing a direct correlation between Pb concentration in water and metal content in fish bodies. The direct correlation between Pb concentration in water and the metal content in fish bodies indicates that gill tissues are exposed to metal ions, affecting their structure and function.

Metals that enter the body through the gills and the liver are released into the circulatory system. While some metals are excreted externally through the kidneys and gills, metal accumulations are stored in tissues or are toxic to target organs. Exposure to Pb through water causes a relatively high accumulation rate compared to metal exposure through feed, because gill tissues are exposed to metal ions, thus affecting gill structure and function. In addition, metal accumulation may depend on the environmental differences between seawater and freshwater (Lee *et al*., 2019).

The results showed that Pb concentration in water correlated with the metal content in milkfish. Treatments with higher Pb concentrations in the rearing tanks showed a significant increase in the metal content of the fish. In comparison, treatments with lower Pb concentration showed decreased metal content. The correlation between Pb concentration in water and the metal content in milkfish aligns with the theoretical understanding that fish absorb metals directly from the surrounding water. The study also mentioned that Pb bioaccumulation in the gills increased significantly after ten days, which supports the idea that exposure duration affects metal accumulation in fish tissues (Abdel-Warith *et al*., 2020). Abdel-Warith *et al*. (2020) conducted the present study, showed that the bioaccumulation of $Pb(NO_3)_2$ in gills increased significantly after ten days, and there was also an increase after 20 d in catfish (*Clarias gariepinus*) exposed to metals at different concentrations. Aquatic organisms have a unique ability to organize important metals to some extent. However, this ability is impaired by continuous exposure to trigger metal accumulation in body organs, which exceeds permissible limits. According to the Class II water quality standard based on Government Regulation of the Republic of Indonesia No. 22 of 2021 Appendix VI; the FAO/WHO Quality Standard for lead in water is 0.08 mg/L. The high lead concentration measured in these internal organs would, almost certainly, contribute to the histopathological alteration found in the fish organs. Fishmay absorb dissolved elements and heavy metals from surrounding water and food, which may accumulate significant concentrations of metals even in waters in which those metals are below the limit of detectionin routine water samples (Abiona *et al*., 2019).

Research results show the environmental impact of heavy metals, such as Pb, and their potential for bioaccumulation in the food chain. Literature shows that heavy metals are released from various sources, including domestic and agricultural

waste, industrial effluents, and mining activities, which can contaminate aquatic ecosystems (Garai *et al.*, 2021). As mentioned in the study results, high lead concentrations measured in fish internal organs may contribute to the histopathological changes in fish organs. The high lead concentrations measured in fish internal organs, as mentioned in the study results, align with the understanding that continuous exposure to metals can lead to the accumulation of metals in organs beyond the permissible limit.

Histology of gills and liver

Gills are essential organs for ion regulation and gas exchange in fishes. Exposure to dissolved lead (Pb) disrupts tissue osmoregulation, causing physiological changes such as lamellae shrinkage. Pb also results in epithelial degeneration and damage such as inflammatory cells, fusion, necrosis, and edema. In juvenile gills of milkfish in treatment A (control), inflammation occurred in response to tissue trauma, with the formation of blood deposits and erythrocytes exiting the blood vessels. The inflammatory response involves regeneration of lost cells, formation of connective tissue, and migration of leukocytes to the necrotic area (Paundanan *et al.*, 2020).

The changes observed in treatment B (0.08 mL.L^{-1}) included edema and necrosis. This is thought to be due to the entry of Pb into the gill lamellae, which causes the formation of lesions. As a result, the walls of the blood vessels become more easily traversed by fluids that quickly escape into the surrounding tissues. Although Pb levels in the body of juvenile milkfish decreased due to exposure to a concentration of 0.08 mL.L^{-1}, necrosis damage still occurred. This may be due to the continuous exposure
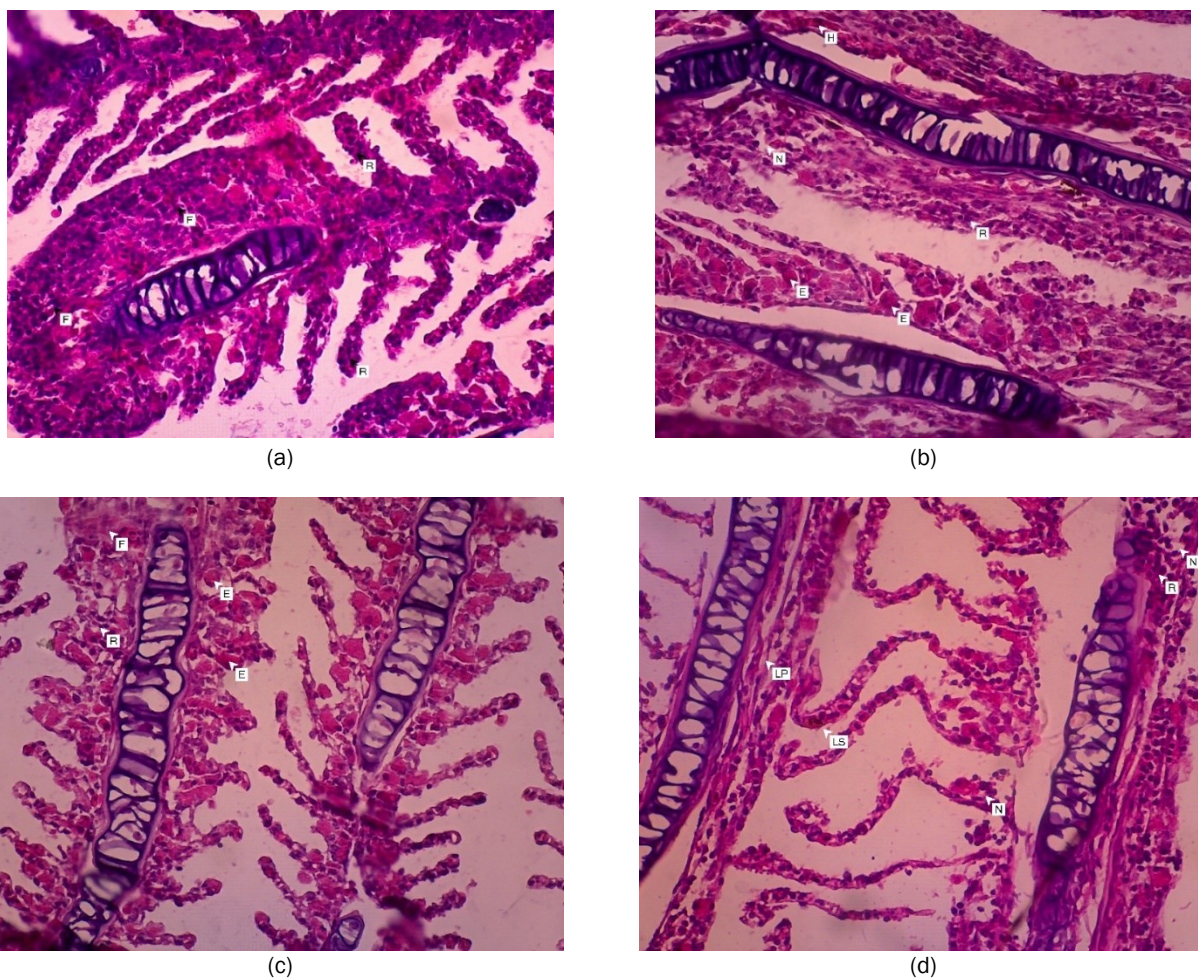

(a)	(b)
(c)	(d)

Figure 2. Gill histology results of juvenile milkfish
Note. (a)Treatment A (control): inflammatory cell accumulation (R) and secondary lamella fusion (F); (b) Treatment B (0.08 mL.L^{-1}) of necritous cells (N), infiltration of inflammatory cells (R), and edema (E); (c) Treatment C (0.8 mL.L^{-1})secondary lamellar fusion (F), cell necrosis (N), inflammation (R); (d) Treatment D (8 mL.L^{-1}) of the gills, consisting of the primary lamella (LP), secondary lamella (LS), and necrosis, appeared to occur in the primary lamella.

dose, indicating that low but chronic doses can cause damage even though Pb levels decrease. The research findings are consistent with Rosmaidar *et al.*, (2017) perspective, stating that the longer the exposure to Pb, the more severe the necrosis becomes. Prolonged exposure to Pb causes necrosis due to the substance inhibiting cellular metabolic processes, thereby preventing cells from producing the necessary energy for survival. Edema results in the swelling of inflamed tissue due to fluid accumulation. Edema is the swelling of cells that occurs due to excessive fluid accumulation in tissues and can lead to the separation of the epithelial layer from the mast cells of the underlying system. This result was reported by Aliza *et al*. (2021), who stated that edema occurs because of increased hydrostatic pressure in blood vessels, which results in leakage of blood plasma fluid into the surrounding tissue. In addition to edema, juvenile milkfish exposed to Pb concentrations experienced necrosis. This might be because juvenile milkfish have been exposed to Pb for a long time. This is by the statement of (Choudhary *et al*., 2019) showed that gill necrosis develops due to prolonged exposure to irritants, including gill epithelial cell necrosis from lead toxicity. Necrosis is an advanced stage of lysis in the tissue where the cells break and the cell nucleus disappears. In addition, necrosis is caused by edema that occurs continuously, resulting in cell death (necrosis) because cells lose the ability to repair existing damage (Kaur and Brraich, 2021).

Treatment C (0.8 ppm) resulted in a secondary lamellar fusion, cell necrosis, and cell infiltration. This lamella fusion was caused by the high Pb concentration. This follows previous research results(Sugiantari *et al*., 2022), namely, lamella fusion found in samples from locations with high heavy-metal concentrations. Lamellar fusion occurs because of a continuous increase in the pathology of hyperplasia. This causes the filling of the space between the secondary lamellae by new cells, which then triggers attachment to both sides of the lamella. Therefore, it can be suspected that milkfish juveniles are poisoned by Pb in 0.8 mL.L^{-1} concentrate, causing hyperplasia to fuse. This fusion penetrates the thickness of the secondary lamella and reduces gas diffusion efficiency is reduced (Gopinathan and Binukumari, 2021).

Treatment D (8 mL.L^{-1}) obtained necrosis. This necrosis also occurred in all treatments, except the control, with varying degrees of organ damage. Cell death was defined as cell necrosis. This was thought to be due to sudden circulatory and ischemic disorders. Complete and sudden ischemia can lead to necrosis. Many cell deaths with limited proliferative properties in the gill lamella can worsen gill respiration function. Damage to the gill structure

causes difficulty in breathing fish, which reduces the oxygen content in the blood and makes it difficult to bind oxygen. Treatment D, with a Pb concentration of 8 mL.L^{-1}, experienced acute exposure with 100% mortality in the first 60 min.

Liver histology after treatment with fish A (0 ppm) revealed the presence of normal hepatocytes and sinusoids. Treatment with B (0.08 ppm) resulted in inflammatory cells, necrotic cells, and hemorrhage. Treatment of the liver parenchyma with C (0.8 ppm) resulted in necrosis, and no normal hepatocytes were found. Inflammatory cell infiltration, necrosis, and hydrofic degeneration were observed in treatment D (8 ppm).

The histological results of juvenile milkfish livers in treatment A (0 mL.L^{-1}.control^{-1}) showed typical liver histology. However, the histological results of other treatments showed significant changes, with signs of inflammation, hemorrhage, necrosis, and worsening of hydropic degeneration, along with increased Pb metal concentrations. These results indicate that exposure to Pb metal has a damaging impact on the histology of the liver, with damage levels positively correlated with higher Pb metal concentrations, which has important implications for understanding the impact of heavy metals on fish health and protection of the aquatic environment.

The histological results of milkfish liver in treatment B (0.08 mL.L^{-1}) showed a significant change in liver tissue. Some striking histological findings include the presence of inflammatory cells or inflammatory cells, indicating that exposure to Pb at concentrations of 0.08 mL.L^{-1} for 30 d has stimulated an ongoing inflammatory response in whitefish livers. This suggests that organisms continue to respond to heavy metal exposure with efforts to address their adverse effects. In addition, necrotic cells are found, which indicate the death of liver cells as well as hemorrhaging or hemorrhage, reflecting damage to blood vessels in the liver tissue. Sustained exposure to heavy metals, such as Pb, can result in significant organ damage and potentially threaten the health and survival of whitefish.

In treatment C with a Pb concentration of 0.8 mL.L^{-1}, the liver parenchyma showed severe necrosis, and no normal hepatocytes were found. This indicates that exposure to Pb metal at a concentration of 0.8 mL.L^{-1} for 30 d resulted in serious liver damage with loss of normal hepatocyte structure and replacement of liver tissue by necrotic cells. This is because the entry of toxic substances can decrease the activity of enzymes that detoxify toxins. The necrosis stage begins with an inflammatory reaction in the liver, in the form of
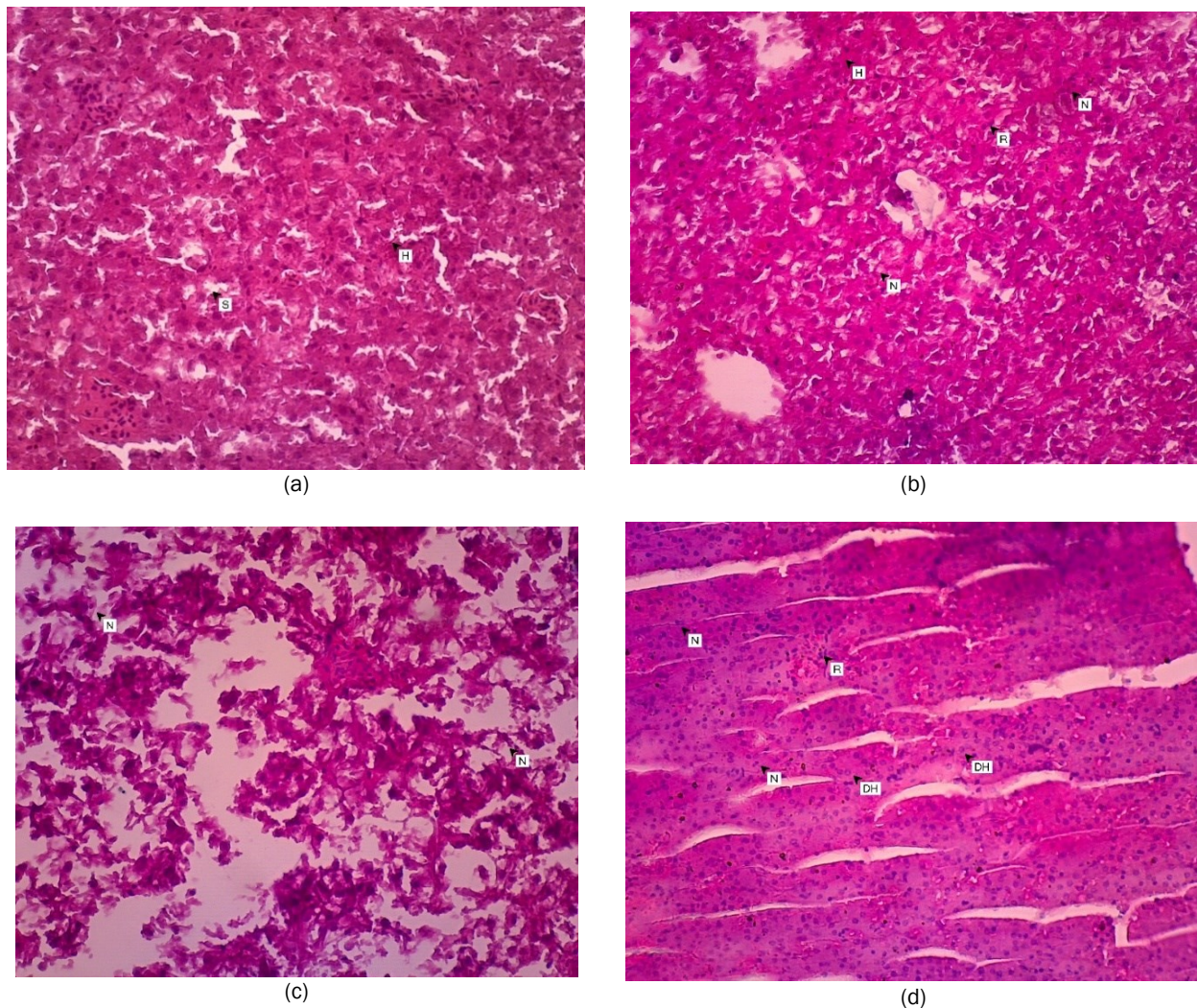
Figure 3. Liver histology results of juvenile milkfish
Note: (a) Treatment A (Control) contains hepatocytes (H) and sinusoids (S); (b) Treatment with B (0.08 mL.L^{-1}) results in inflammatory cell accumulation (R). Necrotic cells (N) and hemorrhagic cells (H); (c) Treatment C (0.8 mL.L^{-1}) of the liver parenchyma showed necrosis, necrotic cells (N), and the absence of normal hepatocytes; (b) Treatment D (8 mL.L^{-1}) included inflammatory cell infiltration (R), cell necrosis (N), and hydrofic degeneration (DH).

hepatocyte swelling. Necrosis is an advanced stage of degeneration, in which hepatocyte cells work more heavily because they have to reabsorb too much material, resulting in cell death. Liver cell death occurs along with rupture of the plasma membrane due to the accumulation of large quantities (Jabbar *et al.*, 2021). In treatment D (8 mL.L^{-1}), histological analysis of juvenile milkfish revealed inflammatory cell infiltration, cell necrosis, and hydrofic degeneration. Exposure to Pb at high concentrations damages this organ, causes more severe inflammation, and induces more serious liver cell degeneration until death in the first 30 min. Degeneration occurs when cells lose their standard cell structure due to influences from inside or outside the cell. The presence of metabolic disorders is characteristic of cell degeneration. This causes intracellular and extracellular accumulation of materials, leading to cell death, and is a sign of the beginning of cell damage caused by toxins (Fahrimal *et al.*, 2016). Histopathological studies on acute exposure of fish to heavy metal mixture helps to diagnosis the impact of heavy metal in the fish tissues. The extent of damage of the gill tissue was proportionate to the dose and duration of exposure of the fish to heavy metal mixture (Gopinathan and Binukumari, 2021)

Growth

The results of the W-Tukey test showed that the absolute weight growth and specific growth rate in treatment A (0 mL.L^{-1}) were significantly different from those in treatments B (0.08 mL.L^{-1}), C (0.8 mL.L^{-1}),

and D (8 mL.L^{-1}). The metal content of Pb treatment A (0 mL.L^{-1}) continued to increase in weight from the beginning to the end of the observation period. Treatment B (0.08 mL.L^{-1}) was also increased from the beginning of observation to the end. However, treatment C (0.8 mL.L^{-1}) at the beginning of day 20 increased, but on the 30th day, there was a decrease in body weight.

Juvenile milkfish growth, which includes the weight gain process, is associated with Pb metal content in the body. As shown in Figure 4, the growth of juvenile milkfish varied during the maintenance period. Exposure to Pb metal at different concentrations showed that exposure to Pb metal showed a significant effect on absolute growth and specific growth rate on the maintenance of juvenile milkfish ($P<0.01$).

The results showed that exposure to Pb metal had a significant impact on the growth of young milkfish. In the observation, the absolute growth of fish weight decreased as the concentration of Pb exposure increased. Fish not exposed to Pb metal showed significant growth, with high absolute weight (2.90±1.03 g), while those exposed to high Pb concentrations (8 mL.L-1) showed no absolute weight growth.

These findings are consistent with the literature highlighting the impact of Pb toxicity on fish growth. Pb exposure is known to cause oxidative stress, organ damage, and changes in blood biochemical profiles, significantly disrupting the body's metabolism. Disturbances in the metabolic rate of the fish body may result in the diversion of energy allocation for growth and reproduction to allocation for repairing tissues damaged by heavy metal exposure (Balali-Mood *et al.*, 2021). Heavy metals can also inhibit fish growth. Lead toxicity (Pb) can also influence the growth rates. The longer the lead exposure and the higher the lead concentration, the lower is the growth rate.

A related study by Ko *et al.* (2019) showed that exposure to hexavalent chromium significantly inhibited the growth of other fish, highlighting the negative effects of heavy metals on the growth of aquatic animals. Exposure to heavy metals, including Pb, increases the accumulation of heavy metals in tissues, which can lead to behavioral disorders, decreased growth, and increased mortality. Growth performance is a factor that reflects environmental toxicity in fish, and even low concentrations of heavy metals have adverse effects, triggering physiological changes, such as growth and metabolism, and reducing health and survival rates.

Table 2. Average absolute growth rate and specific growth rate of juvenile milkfish (*C. chanos*) presented by Pb

Pb concentration (mL.L^{-1})	Absolute weight growth (g) ± SD	Specific weight growth rate (%/day) ± SD
A (0)	2.90±1.03[b]	0.10±0.03[b]
B (0.08)	1.34±0.27[a]	0.04±0.01[a]
C (0.8)	0.76±0.22[a]	0.03.±0.01[a]
D (8)	0.00±0.00[a]	0.00±0.00[a]

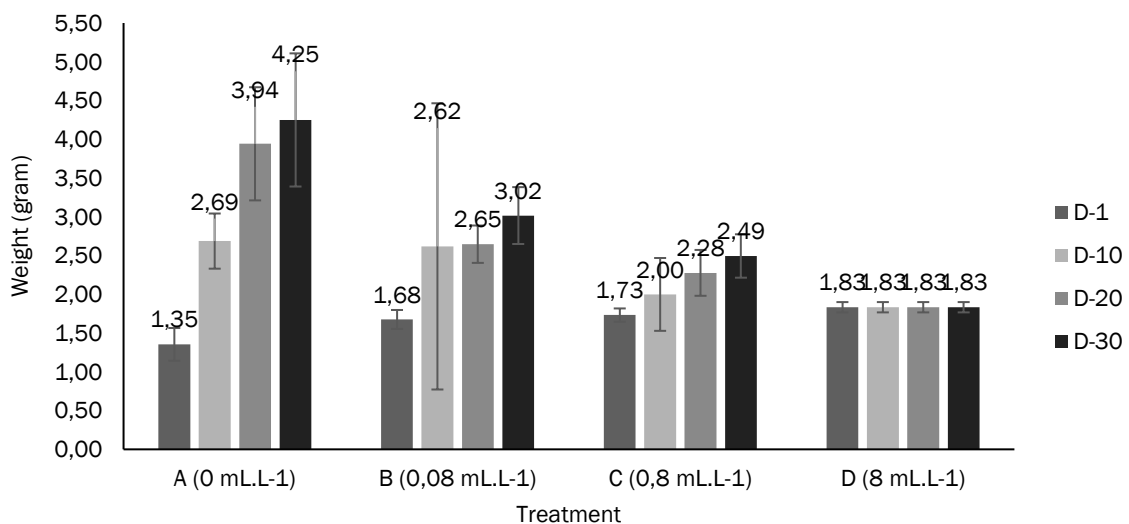Ket.: Different letters in the same column indicate a significant effect ($p < 0.05$)

Figure 4. Weight gain of juvenile milkfish (*C. chanos*) during observations

In addition, the results of this study showed that the reduced growth of Pb-exposed fish was also associated with a decline in overall physical condition. Neurological, respiratory, and oxidant-antioxidant balance disorders are some of the negative effects of Pb exposure, as Balali-Mood *et al.* (2021) suggested. Absolute weight growth and specific weight growth increased in treatment A (0 mL.L^{-1}) because the control had an excellent response to food, so the growth rate increased, whereas in treatments B (0.08 mL.L^{-1}) and C (0.8 mL.L^{-1}), the growth rate decreased. The increase in absolute and specific weights that occurs during fish growth is thought to be due to lead (Pb) accumulation in meat and other organs. According to some studies, Pb accumulates to a greater extent in the gills and the liver. The concentration of heavy metals in meat is lower than in the gills and liver. In aquatic ecosystems, Pb can be easily absorbed into a fish's bloodstream and accumulate in organs Lead (Pb) accumulated more in the gills. Heavy metal content is usually lowest in meat and highest in the gills. This is by the physiological role in fish metabolism where the tissue attacked by heavy metals is one of the tissues that play an active role in metabolism (Sheikhzadeh and Hamidian, 2021).

Survival Rate

Based on the survival data obtained during maintenance, significant changes were observed. Treatment A (0 mL.L^{-1}) on the tenth day (D2) decreased by 3.33%, the tenth day by 6.67%, and the tenth day by 2.22%. Treatment B (0.08 mL.L^{-1}) on the tenth, twentieth, and thirtieth days decreased by 15.56%, 7.78%, and 23.33%, respectively. Treatment C (0.8 ml/L^{-1}) on the tenth, twentieth, and thirtieth days decreased by 14.44, 32.22, and 32.22%, respectively. In contrast, treatment D (8 mL.L^{-1}) resulted in 100% mortality on the first day.

The results showed that the Pb concentration had a natural effect on the survival of juvenile milkfish. Survival continued to decrease with increasing Pb concentrations ($P<0.05$). The survival rate of milkfish at a concentration of A (0 mL.L^{-1}) reached 87.78%, indicating that lead-free environmental conditions support the optimal growth and survival of milkfish. At a B concentration (0.08 mL.L^{-1}), milkfish survival decreased significantly to 53.33%. These results suggest a negative impact of Pb exposure, although the Pb concentrations were still relatively low. The decrease in survival at this level may be related to the response of the immune system and organism to the stress caused by heavy metals. At C concentration (0.8 mL.L^{-1}), the milkfish survival rate dropped to 21.11%, indicating a more significant impact due to higher lead concentrations. At these concentrations, the effects of Pb toxicity on fish became more noticeable, leading to a significant decrease in survival. The organism may experience damage to vital organs, such as the liver, kidneys, and the respiratory system. Mortality in treatment C (0.8 mL.L^{-1}) increased on day 11. The toxic effects of heavy metals in fish are multidirectional and manifest as changes in the physiological and chemical processes. At the D concentration (8 mL.L^{-1}), milkfish survival reached 0.00% on the first day of rearing, indicating that all fish in this group died after exposure to very high lead concentrations. This confirms the detrimental effects of extreme Pb exposure on whitefish survival.

The results of the observations with high concentrations in treatment D (8 mL.L^{-1}) indicated acute exposure. Exposure to high concentrations of Pb rapidly damages and disrupts the biological functions of milkfish, causing death within a short time. Exposure to this high concentration significantly affected juvenile milkfish within approximately 60 min. Such rapid mortality indicates a very high level of Pb toxicity in milkfish and the possibility of very rapid damage to vital organs and systems due to such exposure. Treatments B (0.08 mL.L^{-1}) and C (0.8 mL.L^{-1}) showed clinical symptoms after lead exposure. The clinical signs observed included behavioral changes, such as irregular swimming movements and shock-shock reactions, as well as the tendency of the fish to be on the surface of the water. In addition, the frequency of continuous movement of the operculum occurs with wider openings, followed by periods of inactivity and loss of reflex response in fish. This reaction occurs because of the effect of Pb on the central nervous system, disrupting the cell integrity and function of juvenile milkfish body tissues to exceed tolerance limits, eventually leading to direct death.

Table 3. Average survival of juvenile milkfish (*C. chanos*) exposed to Pb with various concentrations

Pb concentrations (mL.L^{-1})	Survival Rate (%) ± SD
A (0)	87.78±5.1[d]
B (0.08)	53.33±3.1[c]
C (0.8)	21.11±5.1[b]
D (8)	0.00±0.0[a]

Note: Different letters in the same column indicate a significant effect ($P< 0.05$). The results of the variety analysis showed that Pb concentration had a significant influence on the juvenile survival of milkfish ($P< 0.05$).
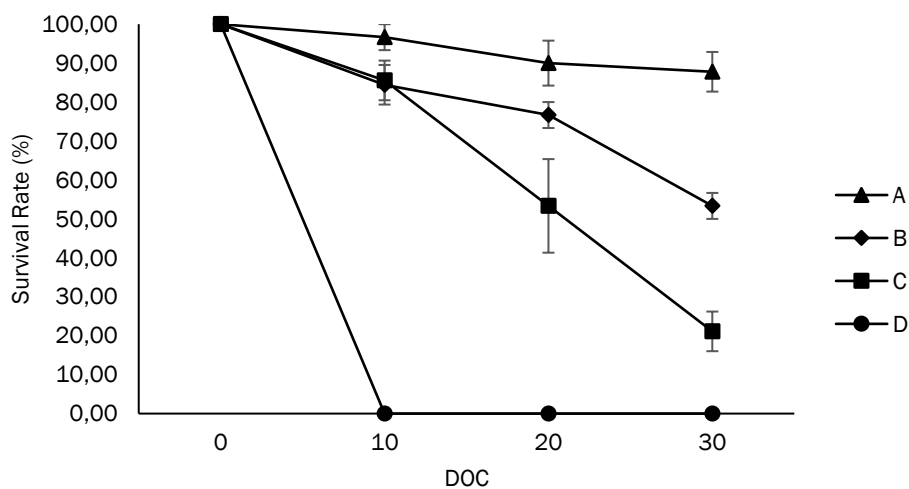
Figure 5. Survival of juvenile milkfish (*C. chanos*) during observation

Juvenile milkfish in treatments B (0.08 mL.L^{-1}) and C (0.8 mL.L^{-1}) showed clinical symptoms following exposure to Pb. The clinical symptoms observed included changes in behavior, such as irregular swimming movements and reactions indicating surprise, along with the tendency of the fish to be on the surface of the water. In addition, continuous operculum movement occurs with a wider opening, followed by periods of inactivity and reduced reflex responses in fish. This reaction occurs because of the impact of Pb on the central nervous system, disrupting cell integrity and the function of body tissues in juvenile milkfish to exceed the established tolerance threshold. Ultimately, this condition results in a gradual death.

The results showed significant changes in the survival of milkfish exposed to Pb metal at different concentrations. Fish survival decreased significantly as the concentration of Pb exposure increased, with the highest survival in the group without metal exposure and the lowest survival in the group exposed to the highest concentration of Pb (8 mL.L-1). This change in milkfish survival is consistent with literature findings indicating that exposure to Pb metal can cause significant toxic effects on aquatic organisms. Some of the dead fish were found to be curled up, with symptoms such as swim-out bubbles, bloody gills, blackened kidneys, and pale liver, as well as excess mucus. This is consistent with previous studies showing detrimental effects on developmental morphogenesis and bone calcification, which in turn can cause deformities in tilapia (*O. niloticus*) (Hassanain *et al.,* 2012).

Chronic exposure to Pb can also produce sclerosis and caudal atrophy in trout, as well as cause abnormalities in the lower jaw, cephalic region, operculum, axial skeleton, and fins in salmon (Esin, 2015). These conditions indicate impaired growth and health of fish in response to Pb metal exposure. Overall, the results showed that exposure to Pb at different concentrations resulted in decreased survival of milkfish. The implication is that water quality management and control of exposure to heavy metals, especially Pb, are critical in the context of fish farming to reduce the risk of toxicity and improve the sustainability of aquaculture production.

Conclusion

Metal accumulation in milkfish exposed to various concentrations shows significant accumulation of metals in the body. Treatment A (control) decreased the metal content until it was not detected. Treatment B (0.08 mL.L^{-1}) also decreased metal levels in the body to 0.073. While the C (0.8 mL.L^{-1}) and D (8 mL.L^{-1}) treatments increased to 3,207 mg.g^{-1} and 6,813 mg.g^{-1}, respectively. Damage to the gills and liver of fish exposed to metal with different concentrations showed significant damage. In the juvenile gills of milkfish treatment A (control) found accumulation of inflammatory cells, and secondary lamella fusion, treatment B (0.08 mL.L^{-1}) found necrotic cells, inflammatory cell infiltration, and edema, treatment C (0.8 mL.L^{-1}) found secondary lamella fusion, necrosis cell, and inflammation, while treatment D (8 mL.L^{-1}) necrosis occurred in the primary lamella tumor. While in the liver Treatment A (Control) hepatocytes and normal sinusoids, treatment B (0.08 mL.L^{-1}) found accumulation of inflammatory cells, necrotic sel, and hemorrhage. Treatment C (0.8 mL.L^{-1}) of liver parenchyma underwent necrosis and no normal hepatocytes were found. While treatment D (8 mL.L^{-1}) there is infiltration of inflammatory cells, cell necrosis, and hydrofic degeneration.

Acknowledgement

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